PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WA/47750	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/US2004/025370	International filing date *(day/month/year)* 05.08.2004	Priority date *(day/month/year)* 05.08.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant CHILDREN'S HOSPITAL MEDICAL CENTER et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.06.2005	13.12.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Reuter, U Telephone No. +31 70 340-1036

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2004/025370

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-23 as originally filed

   Claims, Numbers

   1-27 received on 06.06.2005 with letter of 02.06.2005

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

   * *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4,6,9-16,18,20-26
	No:	Claims	5,7,8,17,19,27
Inventive step (IS)	Yes:	Claims	1-4,20-26
	No:	Claims	5-19,27
Industrial applicability (IA)	Yes:	Claims	1-27
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V.

1 The following **documents** are referred to in this communication:

D1 : WOO J G ET AL: "The -159 C->;T polymorphism of CD14 is associated with nonatopic asthma and food allergy" JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 01 AUG 2003 UNITED STATES, vol. 112, no. 2, 1 August 2003 (2003-08-01), pages 438-444, XP002310373 ISSN: 0091-6749

D2 : ASSA'AD AMAL H ET AL: "Analysis of the R130Q IL-13 polymorphism in patients with food allergy" PEDIATRIC RESEARCH, vol. 49, no. 4 Part 2, April 2001 (2001-04), page 11A, XP008040169 &; ANNUAL MEETING OF THE PEDIATRIC ACADEMIC SOCIETIES; BALTIMORE, MARYLAND, USA; APRIL 28-MAY 01, 2001 ISSN: 0031-3998

D3 : RISMA KIMBERLY A ET AL: "V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 AUG 2002, vol. 169, no. 3, 1 August 2002 (2002-08-01), pages 1604-1610, XP002310372 ISSN: 0022-1767

D4 : LIU X ET AL: "Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4rA in German children: The German Multicenter Atopy Study" JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 01 AUG 2003 UNITED STATES, vol. 112, no. 2, 1 August 2003 (2003-08-01), pages 382-388, XP002310374 ISSN: 0091-6749

D5: WOO J G ET AL: "-159 C to T polymorphism of CD14 is associated with non-atopic asthma and food allergy." JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 111, no. 2 Abstract Supplement, February 2003 (2003-02), page S127, & AAAAI 60TH ANNIVERSARY MEETING; DENVER, CO, USA; MARCH 07-12, 2003 ISSN: 0091-6749

D6: Mitsuyasu et al.: "ILE50VAL VARIANT OF IL4RALPHA UPREGULATES IGE SYNTHESIS AND ASSOCIATES WITH ATOPIC ASTHMA" NATURE GENETICS, NEW YORK, NY, US, vol. 19, 1998, pages 119-120,

XP000918629 ISSN: 1061-4036

2 **NOVELTY** (Art. 33(2) PCT)

2.1 The applicant showed, in contrast to what is specified in the Embase abstract added to the citation of D1, that D1 was available online not before 09.08.2003. The print version of the article was dispatched on 08.08.2003 and also the Embase database abstract was published after the priority date (05.08.2003) of the current application. Consequently D1 is regarded as not being part of the state of the art. However the relevant technical features of D1, namely that the TT genotype of the -159 C to T polymorphism of CD14 is linked to food allergy as well as to non-atopic asthma is also disclosed in D5.

2.2 D5 discloses that the -159 C->;T polymorphism of CD14, especially the TT genotype, is associated with food allergy. This correlation between said genotype and said allergy directly and unambiguously reflects the diagnostic application as claimed in independent method claims 5,7 and 27. The genetic marker per se as claimed in claim 19 is disclosed in D5.

2.3 D4 discloses the combined analysis of the IL-13 allele Q130 and the IL4-R alpha variant V75 as genetic markers (p. 385). Also the combined analysis with the IL4-R alpha variant R576 is disclosed (p 386, second par.) The document discloses all technical features of the independent claim 17 relating to combinations of these markers per se, that are suitable to detect the propensity to food allergy.

2.4 In the light of the cited prior art claims 5,7,8,17,19 and 27 do not meet the requirements of novelty of Art. 33(2)PCT.

3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 Regarding the subject matter of claim 6 D5 is regarded as closest prior art. D5 discloses the association of the TT genotype of the -159 position of the CD14

promoter with food allergy (abstract).

3.2 Claim 6 differs from D5 that in order to determine an individuals propensity to food allergy a SNP marker in a second different gene is determined e.g. the SNP marker responsible for the QR genotype of the R130Q variant of the IL-13 gene.

3.3 The technical effect of the difference seems to be a higher significance of the association to food allergy that is detected with the help of the markers used in combination.

3.4 The problem to be solved is thus to provide a method of determining an individuals propensity to food allergy with an increased significance of the markers analysed.

3.5 D2 discloses that the IL-13 R130Q polymorphism has higher frequency in individuals with food allergy (s. abstract).

3.6 Confronted with the problem of having to provide a method to determining an individuals propensity to food allergy with an increased significance the person skilled in the art would combine the teachings D5 and D2 and would analyse both markers that are linked to food allergy and thus arrive at the claimed method. The person skilled in the art would do this without the exercise of inventive skill. The analysis of a plurality of genetic variants in order to predict a phenotype is a generally known standard procedure (e.g. as it is done in haplotype analysis (see e.g. D3 , tab. 4, p. 1606 last par.). The combined analysis of the two mutations disclosed in the documents D5 and D2 that are associated with food allergy is regarded as a routine experimental optimization the person skilled in the art would select in accordance with the circumstances, without the exercise of an inventive skill in order to solve the problem posed. Furthermore only two of the possible combinations of markers claimed, seem to be significantly linked to food allergy (see description, p. 4 l. 3-14). Consequently claim 6 does not fulfil the requirements of inventive step of Art. 33(3) PCT.

3.7 The same reasoning applies mutatis mutandis to the subject matter of independent method claim 11 that also relates to the analysis of two loci in order to determine the

propensity to food allergy.

3.8 Also dependent claims 9,10,12-16 and 18 do not appear to contain any additional features which, in combination with the features of any claim to which it refers, meet the requirements of the PCT in respect of inventive step. The technical features of said claims merely describe some of several straightforward possibilities and routine experimental optimization procedures from which the person skilled in the art would select in accordance with the circumstances, without the exercise of inventive skill, in order to solve the problem posed.

3.9 Regarding the subject matter of claim 2 D5 is regarded as closest prior art. D5 discloses the association of the TT genotype of the -159 position of the CD14 promoter with food allergy (abstract).

3.10 Claim 2 differs from D5 that in order the determine an individuals propensity to food allergy two further alleles are determined. Whereas the additional determination of the presence of the R130Q variant of the IL-13 gene is not regarded as being inventive (see above), the additional determination of the presence of the V75 allele of the IL-4Ralpha gene is regarded as involving an inventive step.

3.11 The technical effect of the difference seems to be a higher significance of the association to food allergy of the markers used in combination.

3.12 The problem to be solved is thus to provide a method of determining an individuals propensity to food allergy with an increased significance of the markers analysed.

3.13 D4 discloses the combined analysis of the R130Q variant of the IL-13 gene and the presence of the V75 allele of the IL-4Ralpha in patients with increased IgE levels and atopy (abstract). D6 discloses the association of the V75 variant as being responsible for upregulating IgE levels and as being associated with atopy. The food allergy analysed in the underlying application is IgE mediated (see p. 7 l. 4 of the description). Nevertheless, the person skilled in the art would not additionally use the V75 variant, that is known to be generally linked to atopy and increased IgE-levels as a specific marker for food allergy, without the exercise of inventive skills. The

additional determination of the presence of the V75 allele of the IL-4Ralpha gene in a method of determining an individuals propensity to food allergy is neither disclosed nor taught by the cited prior art. Additionally there would be no reasonable expectation of success that the V75 allele disclosed in D6 would function in combination with known food allergy markers as genetic marker for food allergy. Consequently independent claim 2 is regarded as meeting the requirements of the PCT with respect inventive step (Art. 33(3) PCT).

3.14 For the same reasons mutatis mutandis also the subject matter of independent claims 1 and 25, that as well relate to the determination of three variants (in two two-locus combinations in claim 1) including the VV genotype of I75V at IL-4Ralpha, and the subject matter of claim 26, that relates to the detection of the VV genotype of I75V at IL-4Ralpha in combination with the TT genotype at position -159 of CD14 in order to detect food allergy, as well as the corresponding independent product claims 20 and 23 are regarded as meeting the requirements of the PCT with respect inventive step (Art. 33(3) PCT).

3.15 For the assessment of novelty and inventive steps the wording of the claims like "determining the presence of an allele combination...in at least one cell in an individual" (claim 2) and the variations thereof have been read as "determining the presence of an allele combination...in at least one cell (in the sample obtained) from an individual" (s. claim 5).

3.16 In the light of D1-D6 claims 5-19 and 27 do not fulfil the requirements of inventive step of Art. 33(3) PCT.

CLAIMS

1. A method to determine an individual's propensity to a food allergy comprising determining at least one of IL-4Rα, IL-13, or a CD 14 promoter from at least one cell in the individual wherein an excess of two-locus VV (I75V at IL-4Rα) – QR (R130Q at IL-13) and QR (R130Q at IL-13) – TT (at CD14 – 159 C → T) indicates the individual's increased propensity to a food allergen.

2. A method to determine an individual's propensity to a food allergy comprising determining the presence of an allele combination comprising V75IL-4Rα and Q130IL-13 and T159C → TCD14 in at least one cell in the individual wherein an increase of the allele combination over a control indicates the individual's increased propensity to a food allergen.

3. The method of claim 2 further determining the individual's propensity to eczema.

4. The method of claim 2 wherein the individual is an infant.

5. A method of determining an individual's propensity to a food allergy comprising analyzing at least one cell from the individual to determine a TT (CD14 – 159 C→T) genotype, and determining an increased propensity to a food allergy if the TT (CD14 –159 C→T genotype differs from a control.

6. A method of determining an individual's propensity to a food allergy comprising determining in at least one cell of the individual a single nucleotide polymorphism (SNP) marker in at least two different genes of at least two of I75V IL-4Rα, E400A IL-4Rα, C431R IL-Rα, Q576R IL-4Rα, R130Q IL-13 and –159 C→T CD 14 compared to a control wherein the SNP marker indicates an increased propensity to a food allergy.

7. A method of determining an individual's propensity to a food allergy comprising determining in at least one cell of the individual a single nucleotide polymorphism (SNP) marker in –159 C→T CD 14 compared to a control wherein the SNP marker indicates an increased propensity to a food allergy.

8. The method of claim 7 wherein the SNP is in a TT allele.

9. The method of claim 7 wherein the individual is an infant.

10. The method of claim 7 further indicating the individual's propensity to eczema.

11. A method of enhancing determination of an individual's propensity to a food allergy comprising analyzing at least one cell of the individual for a variant in at least a two-locus analysis to enhance association between the genotype in the individual and the phenotype of a food allergy in the individual.

12. The method of claim 11 determining a three-locus analysis.

13. The method of claim 11 wherein at least one loci is in an atopy-associated genetic variant.

14. The method of claim 11 wherein a variant in at least two of CD 14, IL-4Rα, or IL-13 genes is analyzed.

15. The method of claim 11 wherein a variant in at least two of I75V IL-4Rα, E400A IL-4Rα, C431R IL-4Rα, Q576R IL-4Rα, R130Q IL-13, or –159 C→T CD 14 is analyzed.

16. The method of claim 11 wherein a variant is in a V75 allele of IL-4Rα, Q130 IL-13, and a T allele of –159 C→T CD14.

17. A genetic marker for an individual with a food allergy, the marker comprising a single nucleotide polymorphism (SNP) in at least two of a V75 allele of a IL-4Rα gene, a Q130 allele of a IL-13 gene, and a T allele of a CD14 promoter.

18. The marker of claim 17 wherein the SNP in the T allele of the CD14 promoter is –159 C→T .

19. A genetic marker for an individual with a food allergy, the marker consisting essentially of a –159 C→T polymorphism in a CD14 promoter.

20. A genetic marker for an individual with a food allergy, the marker

comprising VV(I75V)-RQ(IL13 R130Q)-TT(-159C→T).

21. A genetic marker according to claim 20, the marker consisting essentially of VV(I75V)-RQ(IL13 R130Q)-TT(-159C→T).

22. A genetic marker according to claim 20 or 21 wherein the food allergy is a peanut allergy.

23. A genetic marker for an individual with a milk allergy, the marker comprising VV(I75V)-TT(-159C→T).

24. A genetic marker according to claim 23 the marker comprising VV(I75V)-TT(-159C→T).

25. A method for identifying an individual with a peanut allergy comprising screening nucleic acid of the individual for the presence of the combination of markers VV(I75V)-RQ(IL13 R130Q)-TT(-159C→T) and identifying the individual as having a peanut allergy if the combination of markers is present.

26. A method for identifying an individual with a milk allergy comprising screening nucleic acid of the individual for the presence of the combination of markers VV(I75V)-TT(-159C→T) and identifying the individual as having a milk allergy if the combination of markers is present.

27. A method for identifying an individual with a food allergy comprising screening nucleic acid of the individual for the presence of homozygous mutant type TT (-159C→T) marker, and identifying the individual as having a food allergy if the marker is present.

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